Summary and future perspectives
Summary

Coeliac disease is a permanent intolerance to ingested gluten resulting in immune mediated inflammatory damage to the small intestinal mucosa and a subsequent malabsorption syndrome in genetically predisposed individuals. Gluten is a protein complex found in wheat, rye, and barley and treatment of coeliac disease consists of a gluten free diet. Coeliac disease can cause a wide variety of symptoms, including both intestinal (diarrhoea, weight loss, abdominal pain) and extra-intestinal (osteoporosis, anaemia).

The association between coeliac disease and type 1 diabetes (T1DM) has long been established. T1DM is characterized by destruction of the insulin-producing β-cells in the pancreas leading to high blood glucose level outside the physiological range. The condition commonly produces classical symptoms of polydipsia (increased thirst), polyuria (frequent urination), increased fatigue and finally without treatment keto-acidotic coma. Treatment involves insulin therapy either in the form of multiple daily injections or pump therapy, together with calculated carbohydrate intake and frequent glucose monitoring. As a result of chronic hyperglycaemia, a number of complications can occur in the long term, especially of microvascular origin including: diabetic retinopathy (eye damage), neuropathy (nerve damage), and nephropathy (kidney disease).

The prevalence of coeliac disease is rated 6 times greater in adults with T1DM than in the general population. The increased prevalence of coeliac disease in T1DM patients is due to a common genetic background and interplay between environmental and immunological factors. Both diseases have long term consequences, however, the additional long term consequences in case of presence of both disease is unknown.

This thesis concerns several clinical and genetic aspects of patients with both T1DM and coeliac disease. Further, we discuss whether screening for coeliac disease is indicated in patients with T1DM.

In chapter 2 we evaluated common practice of diagnosing coeliac disease in T1DM patients in the Netherlands. We studied 118 patients with both T1DM and coeliac disease identified in the Netherlands and we retrospectively collected data on sex distribution, age of onset of T1DM, age of
coeliac disease diagnosis, type of complaints of coeliac disease, duration of complaints of coeliac disease before diagnosis, family history of coeliac disease or T1DM, comorbidity and HLA-DQ type. We observed a bimodal distribution of the age of diagnosis of coeliac disease in T1DM patients with a peak incidence at the age of 10 and 45 years. Furthermore, we found that a large proportion (48%) of our patients diagnosed with coeliac disease in adulthood reported to have had coeliac disease related complaints over 5 years before coeliac disease diagnosis was established. Our observation suggests that physicians should be more aware of the symptoms and/or the association of both diseases and that screening for coeliac disease is recommended in T1DM patients.

Patients with (undiagnosed) coeliac disease may have weight loss, diarrhoea, abdominal discomfort or osteoporosis, however, as indicated previously, data are sparse concerning the effect of concomitant coeliac disease in T1DM patients. In chapter 3 we investigated the course of glycaemic control at coeliac disease diagnosis and after initiation of a gluten free diet (GFD) in T1DM patients and the prevalence of diabetic complications in T1DM patients with adult diagnosis of coeliac disease. We compared 31 patients with coeliac disease + T1DM with 46 T1DM patients matched for age, gender, T1DM duration and glycosylated haemoglobin A1c percentage (HbA1c levels). HbA1c is used as a marker for average plasma glucose concentrations over the past 2-3 months. We found that the diagnosis of coeliac disease and treatment thereafter with a GFD was not of significant influence on glycaemic control in T1DM patients. Further, we observed a lower prevalence of retinopathy in the T1DM + coeliac disease group compared with patients with T1DM only.

It was hypothesized that Advanced Glycation End products (AGEs) play a role in the lower prevalence of retinopathy in the concomitant presence of coeliac disease in T1DM patients and this was investigated in chapter 4. AGEs are proteins or lipids that become glycated after exposure to sugars and AGEs are the result of the Maillard reaction. Although they may be formed as a result of normal metabolism and aging, their formation is exaggerated in the presence of certain pathologic conditions, e.g. oxidative stress due to hyperglycaemia in patients with diabetes. Not only are AGEs formed in certain pro-atherogenic conditions, also by the ambiguous presence of their receptors (RAGE) on the endothelium, AGEs have been shown to contribute to the development of atherosclerosis. Besides endogenously formed AGEs, it has been demonstrated that exogenously formed AGEs (dietary...
AGE’s) are absorbed by the intestine into the bloodstream and represent a source of chemically active toxins\(^\text{10}\). A previous study from Australia observed lower levels of AGEs in patients with both T1DM and coeliac disease compared to T1DM alone, possibly because of a GFD low in dietary AGEs\(^\text{7}\). We therefore compared AGE levels between 25 patients with T1DM and coeliac disease, 25 T1DM patients without coeliac disease and 25 healthy controls. We measured AGE levels by skin autofluorescence (AF) and serum soluble receptor AGE (sRAGE). Although we could previously detect differences in the presence of microvascular complications, no differences were found in skin AF or sRAGE levels between T1DM patients with or without coeliac disease. We did observe higher skin AF levels in patients with T1DM compared to healthy controls. Therefore, our findings suggest that AGE levels are not responsible for the differences in microvascular complications between patients with T1DM with or without coeliac disease.

Since coeliac disease can only be treated by GFD that may differ completely from a regular diet taken by family and friends, coeliac disease might also affect the quality of life (QOL) in patients with T1DM. In chapter 5 we compared QOL of 57 adult patients with both T1DM and coeliac disease with 57 T1DM patients matched for age, gender and socioeconomic status. Generic QOL scores were compared with data from healthy Dutch controls. In the group of patients with T1DM and coeliac disease, women had a lower score on the subscales social functioning, vitality and mental health compared to men. Comparing patients with T1DM + coeliac disease versus T1DM patients revealed that patients with T1DM + coeliac disease have more worries about social functioning and diabetes related complications. Comparison of patients with T1DM and coeliac disease versus healthy controls revealed that social functioning and general health perception is affected in patients with T1DM and coeliac disease. We therefore conclude that coeliac disease has an additional negative effect on quality of life in patients with T1DM which is an important aspect in the support, follow-up and treatment of these patients. We advocate that special attention should be addressed to this observation during out-patient contact with these patients.

In chapter 6 we explored the genetic differences between individuals with both coeliac disease and T1DM versus those with only one disease. T1DM (n=42) and coeliac disease (n=28) associated Single Nucleotide Polymorphisms (SNP’s) and HLA haplotypes were compared in 543 subjects who
developed T1DM and coeliac disease versus 2,472 patients with T1DM only and 2,223 coeliac disease only patients. Two association analyses were conducted: double autoimmunity versus T1DM and double autoimmunity versus coeliac disease. The CTLA4 and IL2RA loci were more strongly associated with double autoimmunity than with either T1DM or coeliac disease alone. The \textit{HLA-DQ}2.5 haplotype was significantly associated with double autoimmunity relative to T1DM only (OR 1.44, \textit{P} = 0.0003). In clinical use, HLA-DQ typing might only be useful in excluding the possibility of developing coeliac disease in HLA-DQ 2.5 or 8 negative T1DM patients\textsuperscript{6}. In addition, a Dutch study found that HLA-DQ typing in T1DM patients is neither distinctive nor cost-effective in screening for coeliac disease\textsuperscript{11}. Our findings suggest that the impact of genetic risk is based, primarily, on specific alleles and genotypes in the HLA class II region, with some support for two genes (CTLA4 and IL2RA) that may be linked through a common immune pathway. This information might aid in building genetic risk models to identify individuals with either T1DM or coeliac disease who are at high risk of developing double autoimmunity.

In the final chapter of this thesis (\textit{chapter 7}) we provide a review of the present knowledge on the clinical consequences of concomitant coeliac disease in adult patients with T1DM and we discuss whether screening for coeliac disease is indicated in patients with T1DM. This overview shows that a delay in coeliac disease diagnosis is frequently found in T1DM patients and that coeliac disease in T1DM leads more often to a decreased bone mineral density (BMD), more diabetic complications, a decreased QOL and a higher mortality. We propose an algorithm for periodic screening (every 5 years) for coeliac disease in T1DM patients.

Screening is performed by testing antibodies against tissue transglutaminase 2 (TG2A) and in case of positivity a confirmatory small intestinal biopsy is recommended. If coeliac disease is diagnosed, a clinical work-up should be performed consisting of referral to a dietitian, initiation of a GFD, investigation of possible vitamin deficiencies and measurement of BMD. In case of negative TG2A serology should be repeated after 5 years. In case of gastrointestinal complaints with negative TG2A levels another diagnosis should be considered. An earlier diagnosis of coeliac disease might lead to less complications and a better QOL in T1DM patients.
Future perspectives

This thesis reveals novel insights into the clinical and genetic aspects of patients with both T1DM and coeliac disease, yet many areas require further study.

First, there are no effective strategies to prevent or cure autoimmune diseases. Both coeliac disease and T1DM have a strong HLA-association, indicative of the involvement of the adaptive immune system and the presence of autoantibodies is characteristic. In the affected individual with coeliac disease, 4 components interact: gluten, TG2A, HLA-DQ2/8 and T cells\textsuperscript{12}. However, there is a lack of information on environmental factors that might trigger the autoimmune process. In coeliac disease, the HLA DQ association is very strong: approximately 95% of the patients express HLA-DQ2 and the remainder is mostly HLA-DQ8 positive\textsuperscript{13}. Nevertheless, although some 40% of the population in the Western world expresses one or both of these HLA-DQ alleles, only 1% of the population develops CD. This suggests an environmental trigger in the pre-autoimmune process which is currently unknown.

Alterations in the composition of the gut microbiota might play a role in coeliac disease development\textsuperscript{14}. Studies report intestinal dysbiosis in coeliac disease patients; untreated and treated with a gluten-free diet (GFD), compared to healthy controls\textsuperscript{15,16}. A GFD per se influences gut microbiota composition, and thus constitutes an inevitable confounding factor in studies conducted in CD patients. To improve our understanding of whether intestinal dysbiosis is the cause or consequence of coeliac disease, prospective studies in healthy infants at family risk of CD are needed\textsuperscript{17}. In addition, studies have investigated several environmental factors for the development of T1DM (viral infections, lack of breast-feeding after birth and the consumption of cereals) although these factors are not strongly associated with T1DM. The microbiota might play a role since a recent study in humans suggests that the gastrointestinal microbiota tends to reach a more or less stable state proportionally with an infant’s age, whereas children who developed β-cell immunity have a less diverse and stable gastrointestinal microbiota\textsuperscript{18}. More prospective studies are needed to elucidate the microbiota as environmental trigger for both T1DM and coeliac disease.
Second, screening for CD in T1DM fulfils almost all of the WHO criteria for screening (Table 1). An important unanswered question is whether it is of benefit to diagnose and treat asymptomatic coeliac disease in T1DM patients. Does screening and treatment by a GFD outweigh the harms of managing a population already burdened with an established chronic illness? The benefit of an economically and socially difficult GFD in asymptomatic screen-detected CD patients remains controversial\textsuperscript{19,20}. It was observed that asymptomatic patients with CD reported better self perceived health and less concern with their disease prior to dietary modification\textsuperscript{21}. The CD-DIET Study (Coeliac Disease and Diabetes Dietary Intervention and Evaluation Trial) is a multicentre randomized controlled trial in Canada aimed at evaluating the safety and efficacy of a GFD in patients with asymptomatic coeliac disease and T1D over 1 year\textsuperscript{22}. This study will add important data in the discussion about screening for CD in T1DM patients. Furthermore, prospective longitudinal studies are needed to establish a screening interval for CD in T1DM patients.

A recent systematic review of 9 longitudinal cohort studies involving 11 157 children and adolescents with 587 cases of biopsy-proven CD found that 79% of patients with coeliac disease and T1DM were diagnosed with coeliac disease within 5 years of T1DM diagnosis\textsuperscript{23}. Therefore, they recommend no screening after 5 years of T1DM diagnosis, however, longitudinal studies investigating this, especially in adults, are lacking. Another question with regards to screening for CD in T1DM patients is how to detect overt CD as a recent study has shown that TG2A levels decrease in about 40% of children with T1DM\textsuperscript{24}. Finally, studies are needed which investigate whether testing for CD in T1DM patients is cost-effective.

<table>
<thead>
<tr>
<th>WHO criteria</th>
<th>CD as a candidate for screening in T1DM patients\textsuperscript{25-29}.</th>
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<tr>
<td>That the disease is common and well defined</td>
<td>CD occurs in approximately 6% of the patients with T1DM (25)</td>
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<td>Screening tests are simple, safe and accurate</td>
<td>TG2A screening offers high sensitivity and specificity (26)</td>
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<td>The screening test should be culturally acceptable</td>
<td>Screening seems to be culturally accepted in most parts of the world</td>
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<tr>
<td>Treatment is available</td>
<td>GFD offers symptomatic relief and will lead to mucosal healing (27)</td>
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<td>Clinical detection is difficult</td>
<td>The clinical picture of CD varies and a delay in diagnosis is frequent (28)</td>
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<tr>
<td>If undiagnosed and untreated the disease will lead to severe complications</td>
<td>Symptomatic patients will develop complications (29)</td>
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<tr>
<td>Testing and treatment is cost effective</td>
<td>Studies in screen detected, asymptomatic, CD in T1DM patients with long term follow-up are lacking</td>
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<td></td>
<td>No studies in T1DM patients are performed investigating this item</td>
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2 Daneman D. Type 1 diabetes. Lancet 2006; 11;367(9513):847-58


